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Publications:

1. Studies on Malate Dehydrogenases and Aspartate Aminotransferases from Neurospora crassa. G. B. Kitto, M. E. Kottke, L. H. Bertland, W. H. Murphey, and N. O. Kaplan. Arch. Biochem. Biophys., in press.

2. Kinetic Studies of Dogfish Liver Glutamate Dehydrogenase with Diphosphopyridine Nucleotide and the Effect of Added Salts. L. Corman and N. O. Kaplan. J. Biol. Chem. 242, in press.

3. Purification of Arginine Kinase from Lobster and a Study of Some Factors Affectings Its Reactivation. S. L. Blethen and N.O. Kaplan. Biochemistry (1967), in press.

4. Significance of Substrate Inhibition. N. O. Kaplan, J. Everse, and J. Admiraal. Ann. N. Y. Acad. Sci., in press.

5. Stimulation of Salivary Secretion by a Factor Extracted from Hypothalamic Tissue. S. E. Leeman and R. Hammerschlag. Endocrinology, in press.

Work Accomplished

Kinetic studies have been made with crystalline dogfish liver glutamate dehydrogenase with the diphosphopyridine nucleotides as cofactors. The kinetic constants for the various substrates have been determined. In its sensitivity to guanosine 5'-triphosphate, adenosine 5'-diphosphate, and excess reduced diphosphopyridine nucleotide the enzyme is similar to that derived from other vertebrates. The inhibition by excess reduced diphosphopyridine nucleotide of the initial rate of the aminating reaction was forestalled by the presence of increased ammonium chloride in the reaction mixture. The effect is attributable to the chloride ion. A number of anions have been tested and the order of their effectiveness followed the Hofmeister series: ClO_4^- -> SCN^- > I^- > Br^- > Cl^- > F^-. Peak effectiveness was observed at comparatively low concentrations of salts, varying from 0.045 M ClO_4^- to 0.25 M Cl^-. Further increase in salt concentration led to a reduction in the protective effect of the salts. At non-inhibitory levels of reduced diphosphopyridine nucleotide the same concentrations of salts inhibited the initia velocity but did not alter the extrapolated maximal velocity of the reaction. The presence of added salt minimized the regulatory influenc of the nucleotides guanosine 5'-triphosphate and adenosine 5'-diphosph

Arginine kinase has been crystallized from the tail muscle of the American lobster (Homarus americanus). The preparation appeared to be homogeneous on starch gel electrophoresis at pH 8.6 and on immunodiffusion in agar bel. In the analytical ultracentrifuge, it migrated as a single symmetrical peak with a sedimentation constant of 3.25 S; in 8 M urea, a sedimentation constant of 3.1 S was observed. A molecular weight of 40,000 was calculated from sedimentation equilibrium data. The amino acid composition of lobster arginine kinase was determined; it contained 5 cysteine residues per molecule and no cystine. It can be reversibly inactivated by treating with 8 M urea. The reactivation of arginine kinase was studied by diluting samples from urea into buffer and measuring the increase in enzymatic activity after dilution. Reactivation is promoted by thiols and inhibited by divalent metal ions. Metal ion inhibition can be overcome by thiols or by addition of EDTA. L-arginine or ATP in concentrations sufficient to give almost complete saturation of the enzyme can also promote reactivation in the absence of thiols although they are not so effective as the thiols. ADP is less effective in promoting reactivation than either L-arginine or ATP; arginine analogues do not promote reactivation. Arginine-promoted reactivation is inhibited by metal ions.

A polypeptide that stimulates an increase in the volume and α-amylase activity of saliva within seconds after intravenous injection into anesthetized rats has been detected and partly purified from bovine and rat hypothalamic tissue. This polypeptide, first noticed after fractionation of bovine hypothalamic extracts on G-75 Sephadex and named sialogen, gives a dose-response curve for volume and α-amylase activity of the collected saliva, retains its activity in test rats pretreated with atropine sulfate, phenoxybenzamine, or propranolol, and is still active in hypophysectomized rats. Purification by cation exchange chromatography on sulfoethyl Sephadex C-25 shows that bovine sialogen is a strongly basic substance. If sialogen proves to be of physiological significance, it may function as a neurohormone participating with the nervous system in the regulation of salivary secretion.